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The interference of truncated with normal potassium channel subunits leads to abnormal behaviour in transgenic *Drosophila melanogaster*

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The *Shaker* locus of *Drosophila melanogaster* encodes a family of A-type potassium channel subunits. *Shaker*^{102} mutants behave as antimorphs in gene dosage tests. This behaviour is due to the production of truncated A-channel subunits. We propose that they interfere with the function of their normal counterpart by forming multimeric A-channel structures. This hypothesis was tested by constructing transgenic flies carrying a heat-inducible gene encoding a truncated A-type potassium channel subunit together with a normal wild type dosis of A-type potassium channel subunits. The altered subunit leads at larval, pupal or adult stages to the transformation of wild type into *Shaker* flies. The transformed flies exhibited a heat-inducible abnormal leg shaking behaviour and a heat-inducible facilitated neurotransmitter release at larval neuromuscular junctions. By the overexpression of an aberrant A-channel subunit the normal behaviour of transgenic *D.melanogaster* can be altered in a predictable way.

Key words: potassium channel/P-elements/*Shaker*-mutants

Introduction

Enhanced neuronal excitability is correlated with cellular events underlying associative learning and memory (Abrams and Kandel, 1988). Neuronal excitability is regulated by a great variety of potassium channels. For example, a reduction in K^+ currents in B photoreceptors is observed after a classical conditioning procudure which produces long-lasting changes in the behaviour of the marine mollusc *Hermissenda* (Crow, 1988). In particular, A-type potassium channels are involved in alterations of synaptic efficiency. Reduction in the activity of these channels leads to an increase in presynaptic neurotransmitter release reminiscent of a facilitated neurotransmitter release. The activity of A-type potassium channels is altered in *Shaker* (*Sh*) mutants of *Drosophila melanogaster*.

Sh mutants lack or have impaired transient outward potassium currents (I_A) in nervous and in muscle tissue (Wu and Haugland, 1985; Ganetzky and Wu, 1986; Tanouye *et al.*, 1986; Haugland and Wu, 1989). Therefore, *Sh* mutants are ideally suited to study the molecular basis of the relationship between a behavioural trait and an altered neuronal excitability caused by dysfunctional potassium channels. Recently, it has shown that the *Sh* locus encodes a large transcription unit which expresses multiple A-type potassium channel subunits (Iverson *et al.*, 1988; Kamb *et al.*, 1988; Pongs *et al.*, 1988; Schwarz *et al.*, 1988; Timpe *et al.*, 1988). These subunits are translated from mRNAs which are generated by multiple alternative and differential splicing mechanisms of primary transcript(s). The various *Sh* potassium channel subunits share a common core region which is flanked by variant amino and carboxy-terminal sequences. Mutations in the *Sh* transcription unit obviously lead to non-functional or to aberrant potassium channels. However, genetically mapped *Sh* mutations and the physical map of the *Sh* locus have not been correlated yet. Thus, at the beginning of this study the actual molecular basis of *Sh* mutants was not clear. *Sh* mutants give rise to a dominant phenotype and behave as antimorphs in gene dosage tests. Therefore, it was postulated that the members of the *Sh* potassium channel family form multimeric assembles in order to build functional A-type potassium channels.

This hypothesis was investigated by studying the molecular basis of the *Sh*^{102} mutation which causes a strong dominant Sh phenotype. We show that this phenotype is due to the production of truncated potassium channel subunits. Then we constructed transgenic flies by inserting complementary DNA encoding a truncated Sh potassium channel protein in a heat-shock expression vector and subsequent germ line transformation. The behaviour of transformant flies was studied. The results show that overexpression of one aberrant member of the Sh protein family interferes with the function of wild type A-potassium channels. Accordingly, the behaviour of the transgenic flies is like that of wild type flies before heat-shock, but like that of *Sh* flies after heat-shock. Thus, an altered behavioural phenotype can be produced in a predictable way by altering A-type potassium channel function.

Results

A-type currents are completely absent in the mutants *Sh*^{KS133}, *Sh*^{102} and *Sh*^{M}. Other *Sh*-alleles alter but do not abolish I_A (eg *Sh*^{E62} and *Sh*^{5}) (Wu and Haugland, 1985; Haugland and Wu, 1989). If the effects of *Sh*^{102}, *Sh*^{KS133}, *Sh*^{M}, *Sh*^{5} and *Sh*^{E62} on I_A in larval and adult muscles, and on action potential repolarization in giant axons are compared, a consistent pattern of severity is seen (Tanouye and Ferrus, 1985). Arranged from most to least extreme, the allelic differences in *Sh* defects are: *Sh*^{102} $\geq$ *Sh*^{KS133} > *Sh*^{M} > *Sh*^{5} > *Sh*^{E62}. Gene dosage tests in which a mutant *Sh* allele (*Sh*^{102} or *Sh*^{KS133}) is combined with several doses of the normal gene indicated that *Sh* mutants are antimorphs (ie the abnormal phenotype is still visible, albeit with reduced severity in these aneuploids). Several working hypotheses could be formulated to account for these observations. The

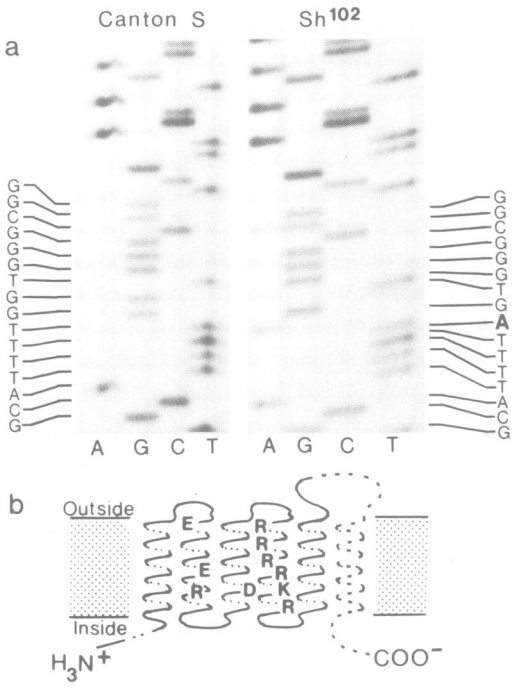

Fig. 1. Molecular basis of the mutation in Sh^{102} flies. (**a**) Sequence of part of exon 15 of the *Sh* transcription unit of Canton S DNA and of Sh^{102} DNA. The sequence corresponds to nucleotides 1258–1273 of *Shβ* cDNA (Pongs *et al.*, 1988). The G to A base change between wild type and mutant DNA is emphasized by a bold A in the Sh^{102} sequence on the right hand side. Wild type DNA of the *Sh* locus has been isolated and sequenced as described previously (Pongs *et al.*, 1988). (**b**) Topological model of a Sh potassium channel subunit (Pongs *et al.*, 1988) inserted into the membrane having the amino- and carboxy-termini on the cytoplasmic side of the membrane. Charged amino acids within the six proposed transmembrane helices are indicated (D-aspartic acid, E-glutamic acid, K-lysine, R-arginine). The broken line in the carboxy-terminal part of the model beginning in front of the sixth membrane spanning segment illustrates the missing part of Sh potassium channel subunits in Sh^{102} mutants.

mutations Sh^{102} or Sh^{KS133} could unbalance the stoichiometry of the family of channel components either by overproducing or underproducing a subset of them. Alternatively, the mutations could yield abnormal subunits that disturb the assembly of functional hetero- and homomultimeric channels.

Since Sh^{102} mutant has the most extreme *Sh* phenotype, its molecular basis was characterized. The Sh^{102} phenotype does not exhibit a tissue or developmental specificity and does not lead to an obvious alteration of *Sh* mRNAs (data not shown). The mutation has been mapped (Timpe and Jan, 1987) proximal to the translocation breakpoint T(X;Y)B55. Therefore and because of the antimorphic behaviour of Sh^{102} mutant, we tentatively located the Sh^{102} mutation within the DNA interval (Pongs *et al.*, 1988) that encodes exons 7–15 which are common to all *Sh* RNAs that have been expressed in *Xenopus* oocytes (Iverson *et al.*, 1988; Timpe *et al.*, 1988; Timpe *et al.*, 1988a). Exons 7–15 have been mapped (Pongs *et al.*, 1988) within 12 kb of *Sh* DNA immediately proximal to the T(X;Y)B55 breakpoint. The restriction map indicated that exons 7–9 are located within *Hin*dIII and exons 10–15 within *Eco*RI–*Hin*dIII restriction fragments. The appropriate restriction fragments of Sh^{102} DNA were cloned and sequenced (Murray, 1983; Maniatis *et al.*, 1982; Sanger *et al.*, 1977). We discovered one base exchange (G to A) (Figure 1a) between wild type and Sh^{102} DNA in the sequence of exon 15. This alters in the Sh^{102} coding sequence, a codon for the amino acid tryptophan (TGG) into a termination amber codon (TAG). Our previous model for A-channel subunits (Pongs *et al.*, 1988) proposed six possibly membrane-spanning helices which are oriented in a pseudosymmetric fashion across the membrane. Thus, the Sh^{102} mutation generates truncated potassium channel subunits lacking the sixth proposed transmembrane spanning helix and the entire carboxy-terminus (Figure 1b).

The *Sh* transcription unit encodes a family of long proteins (L) (~650 amino acids long) (Kamb *et al.*, 1988; Pongs *et al.*, 1988; Schwarz *et al.*, 1988) and a family of short proteins (S) (~350 amino acids long) (Baumann *et al.*, 1987; Kamb *et al.*, 1988; Pongs *et al.*, 1988). The protein structures of all members of the L family are truncated in Sh^{102} flies, whereas the protein structures of S family members possessing only the first three putative membrane-spanning helices (Figure 1b) are not likely to be affected by the Sh^{102} mutation. Since functional A-channels are absent in Sh^{102} flies (Wu and Haugland, 1985), it can be deduced that the short Sh proteins cannot form A-channels by themselves. Also, functional A-channels are not expressed in *Xenopus* oocytes from cRNA encoding S family members (Iverson *et al.*, 1988).

The generation of truncated A-channel subunits provides a molecular basis for the antimorphic behaviour of Sh^{102}. If truncated Sh^{102} proteins are incorporated into a multimeric structure and thus render the A-channel non-functional, the Sh phenotype prevails if even a single Sh^{102} channel subunit is included in the multimer. Thus flies would always exhibit a Sh phenotype when a truncated A-channel subunit is expressed together with wild type A-channel subunits. This prediction was tested by constructing an artificial gene fusion in which a Sh protein-coding region is fused to the heat-inducible promoter of the heat-shock gene *hsp70* (Pelham, 1982; Lis *et al.*, 1983; Craig, 1985). As the *Sh* transcription unit is very large and comprises >120 kb (Schwarz *et al.*, 1988; Pongs *et al.*, 1988, Kamb *et al.*, 1988), *Sh* cDNA was inserted between the heat-shock promoter of *hsp70* and its termination-processing sequences (Figure 2). From the available multiple *Sh* cDNAs, *Shβ* cDNA (Pongs *et al.*, 1988) was selected because it encodes a functional A-channel protein (ShB), according to expression studies in *Xenopus* oocytes (Iverson *et al.*, 1988; Timpe *et al.*, 1988). The second cDNA ($Sh\beta_T$), a *Kpn*I restriction fragment of *Shβ* cDNA, encodes a potassium channel protein (ShB_T) truncated in a way to mimic Sh^{102}. ShB_T protein lacks the last third of the sixth transmembrane helix and the carboxy-terminus of ShB (Figure 2c). Both *Sh* cDNAs were inserted into the *Kpn*I (Asp718) site of the pHT4 derived vector pWH1 (Klemenz *et al.*, 1987; Schneuwly *et al.*, 1987) (Figures 2a and b). The insertion extended the open reading frame (ORF) of $Sh\beta_T$ cDNA by an additional five amino acids (Figure 2c), encoded in the polylinker sequence joining $Sh\beta_T$ cDNA to the termination-processing sequences of the *hsp70* gene. The final constructs, plasmids KB and KB_T, used for germ line transformation are shown in Figures 2a and b. DNA from pKB or pKB_T was coinjected with pπ25.7w.c. helper plasmid into w^- recipient eggs (Klemenz *et al.*, 1987). Several independent, w^+ transformed fly lines were established which had insertions

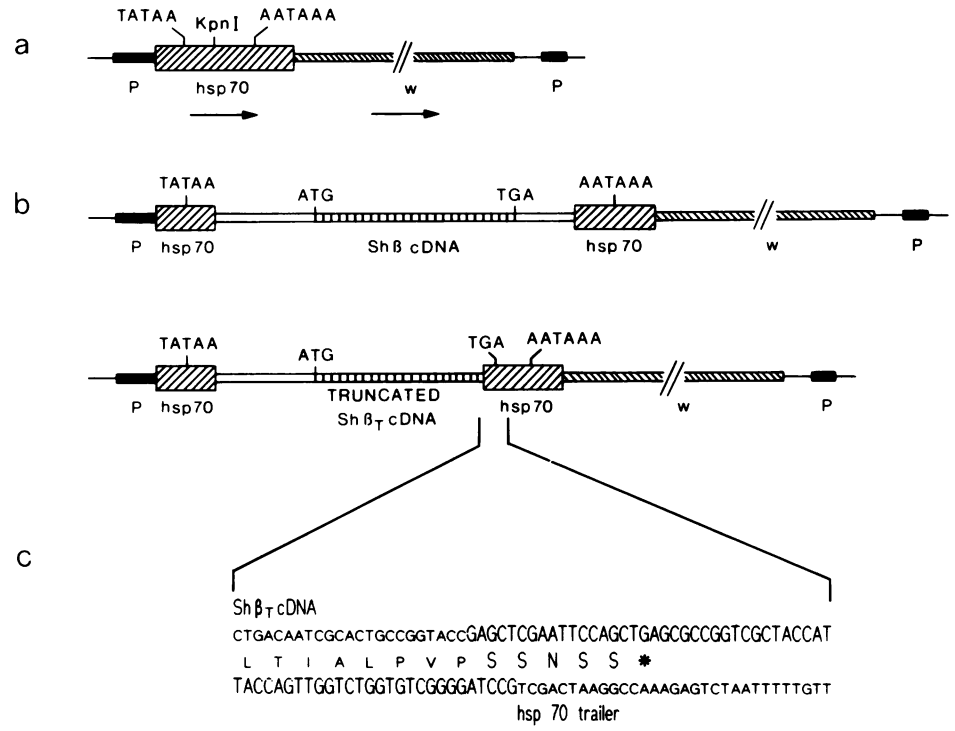

Fig. 2. Structure of heat-shock *Sh* fusion plasmids. (a) Vector plasmid pWH1. This vector was constructed by inserting the *hsp70* promoter and the termination-processing sequences of pHT4 (Schneuwly *et al.*, 1987) into the polylinker of pW8 (Klemenz *et al.*, 1987) vector which contains an artificial *white* (w^+) gene fused to the *hsp70* promoter flanked by the ends of the P-transposon. The unique *Kpn*I site separates the *hsp70* leader from the *hsp70* trailer sequence. (b) *Shß* cDNA (Pongs *et al.*, 1988) was cloned into the *Kpn*I site pWH1, resulting in a fusion of *Shß* cDNA to the *hsp70* leader and expression under the heat-shock promoter. *Shß*$_T$ cDNA was a *Kpn*I fragment of the subcloned *Shß* cDNA. *Kpn*I cuts the ORF (indicated by vertical stripes) at nucleotide 1788. The derived Shß$_T$ protein misses 141 carboxy-terminal amino acids (Val464–Val604). *Shß*$_T$ cDNA was ligated into the *Kpn*I site of pWH1, resulting in an extension of the *Shß*$_T$ ORF by 5 amino acids derived from the fusion to the polylinker. (c) Sequence of the carboxy-terminus of ShB$_T$ protein derived from the fusion of the *Shß*$_T$ *Kpn*I fragment to the *hsp70* trailer sequence. The polylinker sequence is given in large capital letters. The termination codon (TGA) is located 215 nucleotides upstream of the polyadenylation signal AATAAA in the trailer sequence.

on autosomes. Since the *Sh* locus maps on the X-chromosome, transgenic flies with insertions of pKB or pKB$_T$ DNA on the X-chromosome were not used in order to avoid any possible complication in the subsequent analysis of wild type and mutant *Sh* transcripts. Lines KB60, KB$_T$10 and KB$_T$30 had insertions on the second, lines KB10, KB20, KB30, KB40, KB50, KB$_T$20 and KB$_T$40 on the third chromosome. All lines are homozygous viable except for the line KB$_T$20, in which the insertion seems to have induced a lethal mutation. Lines KB20 and KB$_T$40 were used for most of the experiments described below. Other lines showed similar results both in terms of shaking under ether anaesthesia and alteration in synaptic transmission at the larval neuromuscular junction.

To determine whether heat-induction of the expression of the integrated fusion genes induced in Sh phenotype, the leg shaking behaviour of wild type and transgenic *Drosophila* was examined. Under ether anaesthesia wild type flies are immobile or shake their legs only slightly. *Sh* mutants shake their legs vigorously (Kaplan and Trout, 1969). Like wild type *Drosophila* and control w^+ transformants, which only had integrated pHW1 vector DNA, heat-shocked KB transformants did not exhibit a Sh phenotype (Figure 3). This latter result is consistent with the genetic data that duplications of Sh^+ do not lead to a Sh phenotype (Tanouye *et al.*, 1981). KB$_T$ transformants had already without heat-shock, a weak *Sh* like leg shaking activity under ether anaesthesia, probably because the *hsp70* promoter is weakly active at ambient temperature (Steller and Pirrotta, 1985). A strong *Sh* behaviour was induced in KB$_T$ transformants when a heat-shock was applied during the mid to late pupal period and after eclosion (Figure 3). The degree of shaking activity was approximately three times stronger than in KB$_T$ transformants heat-shocked during early pupal development. These results demonstrate that a dominant and persistent Sh phenotype is generated through heat-induced overexpression of ShB$_T$ protein which interferes with the function of wild type *Sh* channel subunits.

Absence or alteration of I_A in *Sh* mutants (Wu and Haugland, 1985; Haugland and Wu, 1989) prolongs presynaptic action potentials (Tanouye and Ferrus, 1985). This causes an increase in synaptic delay and an enhancement in Ca^{2+} influx (Llinás *et al.*, 1982). Thus, at the larval neuromuscular junction of *Sh* mutants synaptic delays are lengthened correlated with excitatory junctional currents (EJCS) of large amplitude (Jan *et al.*, 1977). A similar electrophysiological behaviour was induced in larvae of KB$_T$ transformants. The synaptic delay measured between the presynaptic spike and the EJC in extracellular recordings from larval neuromuscular junctions (Figure 4) was 1.26 ms (± 0.12 SD, n = 10) in wild type larvae before or after heat-shock as compared to 1.68 ms (± 0.41 SD, n = 9) in KB$_T$ larvae kept at 18°C. Probably, the stress induced in larvae during preparation for recordings or the leakiness of the *hsp70* promoter (Steller and Pirrotta, 1985) leads already to a somewhat lengthened synaptic delay in KB$_T$

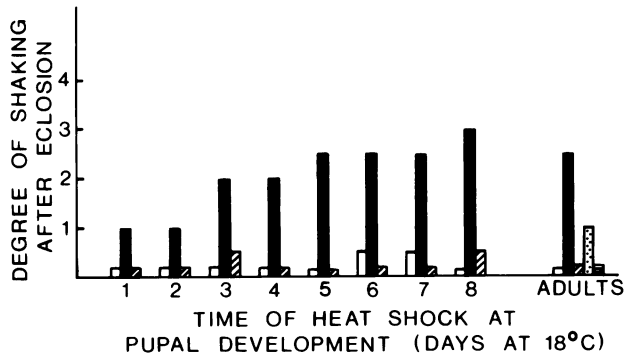

Fig. 3. Induction of Sh phenotype in transgenic flies by heat shock. Flies were allowed to lay eggs at 22°C for 24 h. Cultures were maintained at 18°C. Prepupae were collected in small tubes. They were heat-shocked for 2 h at 29°C at the indicated days of pupal development. After heat-shock pupae were returned to 18°C for continued development. One day after eclosion heat-shocked and w^- control *Drosophilae* were coanaesthetized for examination of leg shaking activity (l.s.a.). Adult flies ('adults') were heat-shocked for 2 h at 29°C one day after eclosion and examined 1–2 h later. L.s.a. was judged by observation of frequency and amplitude of leg shaking. The degree of l.s.a. was classified from weak to very strong as '1,2,3 or 4'. Classifications are based on 20–40 observations (*n*). Open bars—l.s.a. of wild types flies (w^+) heat-shocked during pupal development or one day after eclosion, respectively (n = 20 for each experiment); hatched bars—l.s.a. of w^+ control transformants established after coinjection of pWH1 vector DNA with helper plasmid into w^- recipient eggs (Klemenz *et al.*, 1987) (n = 20 for each experiment); filled bars—l.s.a. of heat-shocked KB_T transformants (n = 30–40 for each experiment i.e. 10–15 flies from each transformant line). Dotted bar indicates the degree of l.s.a. (n = 30) in adult KB_T—transformants maintained at 18°C without heat-shock; striped bar—l.s.a. in adult KB-transformants after heat-shock (n = 30).

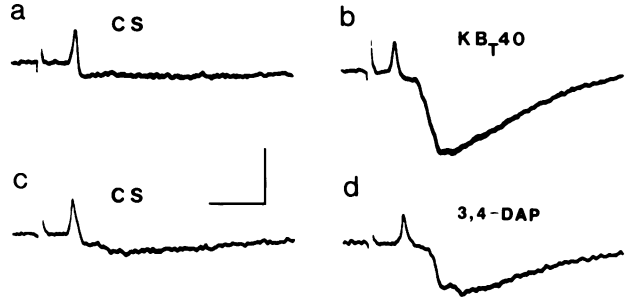

Fig. 4. Extracellular recordings from neuromuscular junctions in the third instar larvae of wild type and transgenic flies. (**a**) Canton S wild-type junction. (**b**) transgenic larvae (KB_T) maintained for 3 days at 30°C before the electrophysiological experiment. Wild type neuromuscular junction examined before (**c**) and after (**d**) application of 25 μM 3,4-diaminopyridine (3,4-DAP). Each trace is the average of 50 events. Calibrations 1 mV and 4 ms. The pre- and postsynaptic currents have been recorded by glass electrodes (i.d 2–3 μm) positioned on neuromuscular junctions under high resolution visual control. The presynaptic current (early biphasic signal) is the derivative relative in time of the action potential at the nerve terminals. The e.j.c. (broad downward deflection) is a faithful representation of the transmitter release process triggered by the entry of Ca^{2+} in the presynaptic terminals. Low $[Ca^{2+}]_o$ was used to minimize the transient Ca^{2+}-dependent K^+ current which is the main action potential repolarizing current in larval muscle and motor endings (Gho and Mallart, 1986).

larvae. After heat-shock of KB_T larvae the synaptic delay was lengthened to 1.91 ms (± 0.33 SD, n = 14). This was accompanied by a 5-fold increase in EJC amplitude (17 out of 25 junctions). To quantify EJCS enhancement in different junctions, their amplitude was normalized to the size of their respective presynaptic spike. EJC amplitude, expressed in multiples of the presynaptic spike, was 0.51 (± 0.42 SD, n = 10) in wild type as compared to 2.15 (± 2.48 SD, n = 17) in heat-shocked KB_T junctions. Application of 3,4-diaminopyridine which blocks I_A (Gho and Mallart, 1986) in motor endings more potently than 4-aminopyridine, induced a lengthening of the synaptic delay (1.96 ms, n = 2) and an increase of the EJC amplitude (3-fold, n = 2) at neuromuscular junctions of wild type larvae similar to the ones induced by heat-shock in KB_T transformants (Figure 4). These results show that heat-induced production of truncated Sh protein eliminates I_A at the neuromuscular junction.

Discussion

Gene dosage experiments (Tanouye *et al.*, 1981; Salkoff, 1983) had shown that the viable *Sh* mutations belong to the antimorph type. Genotypes in which several doses of the normal gene are combined with a single dose of the mutated *Sh* locus, show the mutant phenotype albeit with reduced severity. Recently, we have proposed a molecular basis for the antimorphic nature of *Sh* mutations (Pongs *et al.*, 1988). The mutation causes an abnormal gene product which is assembled with others to form a multimere. As long as the multimeres contain one abnormal gene product, the multimeres cannot function. Our results in this paper provide the final evidence for this proposition. The mutation in Sh^{102} flies causes an abnormal potassium channel subunit which lacks the normally present carboxy-terminal end (Figure 1). Similarly truncated Sh potassium channel subunits do not form active potassium channels, as recently shown by expression studies in *Xenopus* oocytes (Timpe *et al.*, 1988). We conclude that truncated Sh^{102} potassium channel subunits assemble with wild type potassium channel subunits in Sh^+/Sh^{102} heterozygotes and form non-functional multimeres. Since the Sh^{102} mutation belongs to the antimorph type, the assembled multimeres apparently cannot function as potassium channels as long as even one subunit is abnormal. Thus, the molecular basis of antimorphic behaviour of *Sh* mutants is the incorporation of abnormal potassium channel subunits to multimeres. Hence, potassium channels should not consist of one single subunit, but, as proposed previously (Pongs *et al.*, 1988), are built from several subunits into homo- or hetero-multimeric structures.

The Sh^{102} mutation does not discriminate between different members of the Sh L-protein family as all of them are equally affected by the mutation. We have investigated whether a mutation in only one member of the Sh L-protein family causes a Sh phenotype. Accordingly, the Sh^{102} mutation was mimicked for ShB proteins. Transgenic flies were constructed which expressed a truncated ShB_T protein under the control of a heat-shock promoter (Figure 2). It was expected that the heat-induced production of ShB_T protein would interfere with the assembly of wild type *Sh* potassium channels. Similarly to Sh^{102}, assembly of ShB_T protein together with wild type potassium channel subunits would generate non-functional potassium channels. Thus, the introduction of ShB_T constructs into wild type flies should

lead to a heat-inducible dominant Sh phenotype. As predicted, the heat-induced production of truncated ShB_T proteins in transgenic flies carrying the normal dose of the Sh^+ gene invariably leads to a persistent Sh phenotype as shown by the results summarized in Figures 3 and 4. The severity of the Sh phenotype induced in the transgenic flies suggests that the truncated ShB_T protein is assembled not only with ShB proteins into pseudo-homomultimeric structures, but also with other members of the Sh protein family into heteromultimeric structures. However, this proposition has yet to await final proof.

Two alternative, but unlikely explanations are possible to understand the observed dominant Sh phenotype in transgenic flies. If the truncated Sh potassium channel subunits were to be assembled and afterwards would not reach the excitable membrane, a similar antimorph or dominant behaviour would be observed in *Sh* mutants and transgenic flies, respectively. In this case the aberrant ShB_T protein would be metabolized quickly and the heat-induced Sh phenotype should be short-lived. This however, was not observed. The heat-induced Sh phenotype was long-lasting and was observed even 5 days after heat-shock application (Figure 3). Another alternative interpretation of our results might be that homomultimeric ShB_T ensembles are integrated into the membrane. This possibility can be excluded because it would produce loss of function mutants. These mutants do not belong to the dominant type of mutations unless a mechanism operates which regulates the stoichiometry among members of the Sh potassium channel family in the membrane. Such a mechanism seems unlikely because the relative underproduction of ShB protein in KB_T transformants, but not the overproduction of ShB protein in KB transformants produced a Sh phenotype. Taking together the antimorphic nature of Sh^{102} mutation and the dominant heat-inducible Sh phenotype of KB_T transformants strongly suggest that the truncated Sh potassium channel subunits assemble together with wild type channel subunits and integrate as inactive channels into the membrane.

This observation predicts that the carboxy-terminus which is missing in Sh^{102} and in ShB_T proteins is involved in the activation of the channel or in the formation of an active ion pore. Interestingly, the amino-terminus of Sh proteins seems to be involved in the inactivation of the Sh potassium channels. Sequence differences in the amino-termini of the Sh protein family are the basis of profoundly different closing kinetics of Sh potassium channels (Iverson *et al.*, 1988; Timpe *et al.*, 1988).

Changes in behaviour are brought about by alterations of synaptic efficiency (Abrams and Kandel, 1988). As shown in this paper, the heat-induced expression of ShB_T protein changes the normal behaviour of transgenic *D.melanogaster* into that of Sh. This is accompanied by a heat-inducible facilitated neurotransmitter release at neuromuscular junctions (Figure 4). *Sh* mutants produce abnormally prolonged action potentials in the central nervous system. Therefore, synaptic efficiency is probably altered by inactive Sh potassium channels also in the central nervous system. It is envisaged to construct transgenic *D.melanogaster* where a facilitated neurotransmitter release could be induced at specific sites in the central nervous system. The possibility now exists to alter neuronal excitability in a specific manner by overexpression of truncated Sh potassium channels in the brain.

Materials and methods

Sequencing of mutant DNA

Wild type DNA of the *Sh* locus has been isolated and sequenced as described (Pongs *et al.*, 1988). Sh^{102} DNA was digested with *Hin*dIII or with *Hin*dIII–*Eco*RI followed by gel electrophoresis of the resulting fragments on 0.7% agarose gels (Maniatis *et al.*, 1982). Restriction fragments of expected size according to the restriction map of the *Sh* locus (map coordinates +37.5 to +52) were isolated. Isolated fragments were ligated with the corresponding arms of λNM1149 (Murray, 1983). Recombinant phages containing Sh^{102} DNA encoding exons 7–15 of the *Sh* transcription unit (Pongs *et al.*, 1988) were isolated (Benton and Davis, 1977). DNA was subcloned into Bluescript. The dideoxy nucleotide sequencing technique was used for sequencing both strands (Sanger *et al.*, 1977).

Construction of fusion plasmids

Shβ cDNA (Pongs *et al.*, 1988) was subcloned into Bluescript. The subclone was digested with *Eco*RV–*Sma*I creating blunt end fragments. The isolated restriction fragment was cloned into *Asp*718 (an isoshizomer to *Kpn*I) cut vector pWH1 which had been blunt ended with Klenow polymerase. The resulting construct pKB was checked by appropriate restriction enzyme digests. The *Shβ* cDNA subclone was cut with *Kpn*I to generate $Sh\beta_T$ cDNA. The isolated *Kpn*I restriction fragment was cloned into *Kpn*I cut vector pWH1. The resulting construct pKB_T was checked by appropriate restriction enzyme digests and by sequencing as described in the legend of Figure 2.

Transformants

Fusion genes were introduced into the germ line of w^- recipient flies by P-element mediated germ line transformation. DNA of pKB or pKB_T was coinjected with pπ25.7 w.c. helper plasmid into w^- recipient eggs (Klemenz *et al.*, 1987). Transformed w^+ fly lines were established by standard procedures as described (Rubin and Spradling, 1982).

Physiological experiments

Late third instar larvae were pinned out in saline and cut open along the dorsal midline. The internal organs were removed, the nerves innervating the body wall muscles were prepared as previously described (Jan *et al.*, 1977; Gho and Mallart, 1986). The bathing medium and the recording electrode contained (mM) NaCl, 128; KCl, 2; $CaCl_2$, 0.1; $MgCl_2$, 4; Hepes, 5 (pH 7.2). The technique and equipment used for extracellular recordings from neuromuscular junctions maintained at 22°C were described previously (Brigant and Mallart, 1982).

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